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Short communication

Effect of ghrelin on regulation of splenic sympathetic nerve discharge

Sivasai Balivada [a,b,1], Hitesh N. Pawar [a,b,*,1], Shawnee Montgomery [a], Michael.J. Kenney [a,b]

[a] Department of Anatomy and Physiology, Kansas State University, Manhattan, KS 66506, United States
[b] Department of Biological Sciences, College of Science, University of Texas at El Paso, El Paso, TX 79968, United States

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ABSTRACT

Ghrelin influences immune system function and modulates the sympathetic nervous system; however, the contribution of ghrelin to neural-immune interactions is not well-established because the effect of ghrelin on splenic sympathetic nerve discharge (SND) is not known. This study tested the hypothesis that central ghrelin administration would inhibit splenic SND in anesthetized rats. Rats received intracerebroventricular (ICV) injections of ghrelin (1 nmol/kg) or aCSF. Lumbar SND recordings provided a non-visceral nerve control. The ICV ghrelin administration significantly increased splenic and lumbar SND, whereas mean arterial pressure (MAP) was not altered. These findings provide fundamental information regarding the nature of sympathetic-immune interactions.

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1. Introduction

Ghrelin is a peptide hormone that was originally isolated from the gastrointestinal system and identified as an endogenous ligand for the growth hormone secretagogue receptor (GHS-R1a) (Baatar et al., 2011). Subsequent investigations have demonstrated that this peptide can influence a number of physiological systems and responses, including the sympathetic nervous system (SNS) (Lambert et al., 2011; Matsumura et al., 2002). Ghrelin receptors are expressed in the central nervous system, especially in brainstem and hypothalamic sites (Wu et al., 2009), and central ghrelin administration in experimental animals modulates sympathetic nerve discharge (SND) regulation. The intracerebroventricular (ICV) administration of ghrelin has been reported to decrease brown adipose tissue SND in rats (Yasuda et al., 2003), reduce renal SND in rabbits (Matsumura et al., 2002), and produce little effect on renal SND in rabbit offspring from mothers who consumed a normal fat diet compared with enhanced ghrelin-induced renal SND responses in rabbit offspring from mothers fed a high fat diet (Prior et al., 2014). Microinjection of ghrelin in the nucleus of the solitary tract reduces renal SND in rats (Lin et al., 2004). In human subjects direct recordings of efferent muscle sympathetic nerve outflow using microneurography have provided information regarding the effect of peripherally-administered ghrelin on SND regulation. Lambert and colleagues reported that intravenous infusion of ghrelin increased muscle SND and reduced arterial blood pressure (Lambert et al., 2011). These investigators speculated that combined baroreflex-mediated and central neural effects of ghrelin may play a role in mediating the observed sympathoexcitatory response. Krapalis et al., observed a biphasic muscle SND response to intravenous ghrelin; an initial sympathoexcitation associated with reduced arterial blood pressure, followed by a progressive decline of muscle SND towards control levels, despite a sustained reduction in arterial blood pressure (Krapalis et al., 2012). These findings indicate that peripheral ghrelin administration can modulate the level of muscle SND in human subjects, although the role of central neural ghrelin in mediating these responses remains unclear.

A role for ghrelin in modulating immune system function is well-established, primarily as an anti-inflammatory agent and an immunoregulatory hormone (Baatar et al., 2011). Numerous bidirectional pathways provide the foundation for communication between the nervous system and the immune system, and the efferent arm of the SNS plays an important role in mediating neural-immune interactions (Kenney and Ganta, 2014). Physiological activation of splenic SND enhances the expression of splenic cytokine and chemokine genes, an effect that is abrogated by splenic nerve denervation (Ganta et al., 2004), indicating that changes in the level of efferent splenic nerve outflow can influence immune function in a peripheral lymphoid organ.

It is intriguing to postulate that ghrelin may play a role in neuroimmune interactions by influencing the SNS, however, the effect of ghrelin on the level of splenic SND is not known. Given the role of ghrelin as an anti-inflammatory molecule, and the influence of splenic SND activation to influence splenic cytokine gene expression, the first goal of the present investigation was to test the hypothesis that ICV administration of ghrelin would inhibit splenic SND. Important with respect to the role of ghrelin in modulating muscle SND in human

* Corresponding author at: Department of Biological Sciences, College of Science, University of Texas at El Paso, Biosciences Research Building, Room No. 4.216, El Paso, TX 79968, United States.
E-mail address: hnpawar@utep.edu (H.N. Pawar).
[1] These authors contributed equally to this work.

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subjects (Krapalis et al., 2012; Lambert et al., 2011), to date there is no information available concerning the effect of central ghrelin on the level of lumbar SND, despite the fact that this nerve innervates the rat hindlimb and tail (Baron et al., 1988). In this regard, lumbar SND recordings may provide translational significance to previous studies that tested the effect of ghrelin on muscle SND in human subjects (Krapalis et al., 2012; Lambert et al., 2011). Therefore, the second goal was to determine the effect of ICV administration of ghrelin on the level of lumbar SND.

2. Methods

The experimental procedures and protocols were completed in accordance with the American Physiological Society's guiding principles for research involving animals and approved by the Institutional Animal Care and Use Committee at Kansas State University and at the University of Texas at El Paso.

2.1. General procedures

Experiments were completed using male Sprague-Dawley rats (419 ± 15 g). Anesthesia was induced by isoflurane (3–5%) and maintained during surgical procedures using isoflurane (1.25%–1.75%), α-chloralose (80 mg/kg, iv), and urethane (400 mg/kg, ip) (Kenney et al., 2013). Isoflurane anesthesia was discontinued following completion of the surgical procedures. During experimental protocols, maintenance doses of α-chloralose (35–45 mg/kg/h) were administered intravenously and maintenance doses of urethane (200 mg/kg, every 4 h) were administered intraperitoneally. The adequacy of anesthesia during the initial surgical procedures was indicated by the absence of a withdrawal reflex in response to mechanical stimulation of the tail or hind limb. Femoral arterial pressure was monitored using a pressure transducer connected to a blood pressure analyzer. Colonic temperature was maintained at 37.5–37.8 °C during surgical interventions by a temperature-controlled table.

2.2. Sympathetic nerve recordings

Activity was recorded biphasically with a platinum bipolar electrode after preamplification (bandpass filter, 30–3000 Hz) from splenic and lumbar sympathetic nerves. Sympathetic nerves were identified using a lateral approach and dissected free of surrounding connective tissue, and nerve-electrode preparations were covered with silicone gel to prevent exposure to room air. Sympathetic nerve potentials were full-wave rectified, integrated (time constant 10 ms) and quantified as μvolts × seconds (μV·s) (Hosking et al., 2009). SND recordings were corrected for background noise after administration of the ganglionic blocker chlorisondamine (5 mg/kg, iv) or nerve crush.

2.3. ICV injection

Anesthetized rats were placed in a stereotaxic frame. The head was leveled between bregma and lambda, a small hole was made in the skull, and the dura was removed to increase precision of the depth of the injector needle. A Hamilton syringe was loaded onto a Quintessential Stereotaxic Injector (Stoelting Co., Wood Dale, IL), and the needle guided to 1.4 mm lateral from midline, 0.9 mm posterior to bregma, and 3.5 mm below dura. The body weight adjusted ghrelin dose was selected based on previous studies that analyzed the effect of ICV ghrelin injection on SND in animal models (Matsumura et al., 2002; Yasuda et al., 2003). Ghrelin (1 nmol/kg; Phoenix Pharmaceuticals) suspended in artificial cerebrospinal fluid (aCSF) or aCSF alone was administered (10 μL) over 5 min. Fluorescent latex microspheres (50 nm diameter, Lumafluor) were administered immediately before euthanasia to histologically verify that injections were completed in the lateral ventricle.

2.4. Experimental protocols

After completion of surgical procedures, anesthetized rats were allowed to stabilize for 60 min before initiation of experimental protocols. Splenic SND, lumbar SND, mean arterial pressure (MAP), and heart rate (HR) were recorded continuously throughout experimental protocols. Following stabilization, pre-injection values were averaged over a 15 min control period (time 0). At the end of the control period rats were administered ghrelin (1 nmol/kg) or aCSF via infusions in the lateral ventricle. Infusions were completed over a 5 min period followed by a 45 min post-infusion period. At the end of experiments rats were euthanized by an intravenous overdose of methohexital sodium (Brevital®) (150 mg/kg, iv).

2.5. Data collection and statistical analysis

A computer-based ADInstruments Powerlab data acquisition system was used to collect all experimental data. Values are reported as means ± SE. Splenic and lumbar SND data are expressed as percentage change from control values. MAP and HR are reported as the absolute change from control values. Statistical analyses included two-factor (treatment and time) repeated measures ANOVA with one factor repetition (time). Data that demonstrated statistically significant main and interaction effects were further analyzed with Bonferroni *t*-tests. The overall level of statistical significance was $p < 0.05$.

3. Results

The ICV injection sites were histologically confirmed to be in the lateral ventricle in all experiments included in the present study. Fig. 1 shows summarized splenic SND (A) and lumbar SND (B) data recorded before infusion (time 0), at the completion of the ICV infusion period (5 min), and for 45 min post ICV administration of aCSF ($n = 12$) or ghrelin ($n = 8$). Data are presented as change from control levels at 5 min intervals. Splenic and lumbar SND were progressively and significantly increased from control levels after central ghrelin administration ($p < 0.05$), with the magnitude of the sympathoexcitation more robust in lumbar SND. In contrast, splenic and lumbar SND remained unchanged from control levels for the duration of the post-infusion period following ICV aCSF administration.

Fig. 2 shows summarized MAP (A) and HR (B) data recorded before (time 0), at the completion of the ICV infusion period (5 min), and for 45 min after ICV administration of aCSF ($n = 13$) or ghrelin ($n = 12$). MAP did not differ from control levels in either aCSF- or ghrelin-treated rats, or between groups, during the 45 min post infusion period (Fig. 2A). Similarly, HR did not differ from control levels following aCSF and ghrelin infusions, although in ghrelin-treated rats HR tended to increase whereas in aCSF-treated rats HR tended to decrease. The change in HR between the two treatments became significant at 10 min post-infusion and continued until the end of the post infusion period ($p < 0.05$) (Fig. 2B).

4. Discussion

The present results indicate that ICV ghrelin administration in anesthetized rats produces a progressive excitation of splenic sympathetic nerve outflow, a response pattern paralleled by a robust ghrelin-induced lumbar sympathoexcitation. Central ghrelin administration was not associated with significant changes in arterial blood pressure, suggesting that the sympathoexcitatory responses to ICV ghrelin were not secondary to unloading of the arterial baroreceptors. As expected, each of the measured variables remained unchanged from control values in response to the ICV administration of aCSF. These findings support the notion that, under the conditions of the present experiments, administration of ghrelin into the lateral ventricle modulates the central